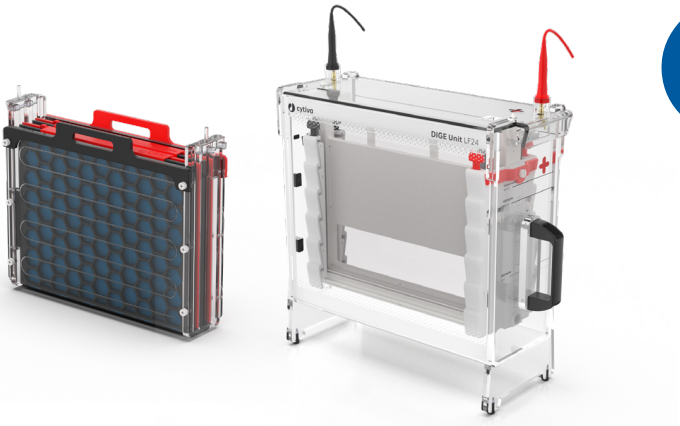
Amersham™ DIGE Unit LF24

Cue Cards



The safety instructions are located in the *Amersham DIGE Unit LF24 Operating Instructions, 29701249* on the translation CD delivered with the product.

All users must read the entire Operating Instructions before installing, operating or maintaining the product.

Scan the QR code or visit *cytiva.com/instructions*. Type `29701249`, and download the Operating Instructions.

For more information go to *cytiva.com*.

1 Introduction

The Amersham™ DIGE Unit LF24 instrument is intended for protein separation with two-dimensional (2-D) electrophoresis. The electrophoresis module is designed exclusively for use with the precast DIGE Gels LF24.

The Amersham Transfer Unit LF24 module is intended for electrotransfer of proteins. The Transfer Unit LF24 module must be used together with the DIGE Unit LF24 tank.

For further instructions go to *cytiva.com*. The user documentation relevant for DIGE Unit LF24 is listed below.

- *Amersham DIGE Unit LF24 Operating Instructions, 29701249*
- *DIBE coverage analysis Instructions for Use, 29657336*
- *2-D Electrophoresis Principles and Methods Handbook, CY14825*

2 Electrophoresis instructions

Step	Action
1	Equilibrate the gels to room temperature.
2	For each gel, heat up one aliquot of sealing solution to 95°C in a heating block.
3	Dilute the anode buffer in the tank: **a.** Pour 125 mL concentrated anode buffer (1 bottle) into a large beaker. **b.** Rinse the bottle with distilled or deionized water, and pour it in the beaker. **c.** Fill up the beaker to 4.5 L with distilled or deionized water. **d.** Pour the buffer into the tank.
4	Dilute the cathode buffer in a separate container: **a.** Pour 800 mL distilled or deionized water into a graduated cylinder. **b.** Pour 2 × 125 mL of concentrated cathode buffer (2 bottles) in a separate container. **c.** Rinse the bottles with distilled or deionized water. **d.** Fill up with distilled or deionized water to 1.2 L.
5	Equilibrate the IPG strip: **a.** Make IPG equilibration buffer in advance: 6 M Urea 50 mM Tris-HCl (pH 8.8) 30% (v/v) Glycerol 2% (w/v) SDS 0.001% (w/v) Bromophenol blue Store aliquots at -20°C. The recommended aliquot volume is 30 mL. **b.** Prior to use, make two IPG equilibration buffers, one containing DTT and the other containing iodoacetamide, respectively.

Buffer	Action
DTT-containing IPG equilibration buffer	For each IPG strip, dissolve 150 mg DTT in 15 mL of IPG equilibration buffer (1.0% w/v).

Step	Action

Buffer	Action
Iodoacetamide-containing IPG equilibration buffer	For each IPG strip, dissolve 375 mg iodoacetamide in 15 mL of IPG equilibration buffer (2.5% w/v).

c. Place the IPG strips in individual tubes or trays and add 15 mL DTT-containing IPG equilibration buffer to each IPG strip.

d. Incubate on an orbital shaker or rocking platform for 15 min.

e. Replace the DTT-containing IPG equilibration buffer with 15 mL iodoacetamide-containing IPG equilibration buffer.

f. Incubate on an orbital shaker or rocking platform for 15 min.

g. Rinse the IPG strip briefly in cathode buffer.

6 Place the IPG strip on top of the gel:

a. Place the IPG strip on top of the gel in the glass cassette. Gently push the plastic backing to move the IPG strip towards the gel upper surface using a thin ruler or a spatula. Make sure the entire IPG strip has contact with the gel and avoid trapping air bubbles between the IPG strip and the gel.

b. Seal the IPG strip in place using 1 mL hot sealing solution. Carefully pipette across the whole length of the IPG strip, taking care not to introduce bubbles.

7 Insert gel cassettes into the gel cassette holder with the notched glass plate facing inward. When running only one gel, fill the other slot with the dummy plate.

8 Place the gel cassette holder in the tank. Make sure that the polarity of the gel cassette holder matches the polarity of the tank.

9 Pour 1.2 L cathode buffer into the inner buffer chamber. Place the safety lid on the tank.

10 Plug in the power leads to the electrophoresis power supply. Make sure that the polarity of the power leads matches the polarity of the electrophoresis power supply.

Step	Action
11	Run the gels with the following run conditions:

Program	Run phase	Voltage (V)[1]	Current (mA)[1]	Power (W/gel)[1]	Time (h)	Cooling
Day run	1	800	400	1	1	N/A
	2	800	400	17	4 to 5	N/A
Fast day run	1	800	400	1	1	Yes[2]
	2	800	400	50	1.5 to 2	Yes[2]
Overnight run	1	800	400	1	8	N/A
	2	80	400	1	9 to 11	N/A

[1] The maximum electrical input for the DIGE Unit LF24 is 800 V, 400 mA, and 100 W.
[2] Use cooled buffer and/or perform the run in the cold room.

12 Stop the electrophoresis run when the bromophenol blue-front reaches the end of the gel. The front can be run off the gel if needed.

13 Scan the gels. Keep the gels in the glass cassettes during scanning.

Note:
To minimize spot diffusion the gels should be scanned as soon as possible.

14 Optional: Add a paper tissue soaked in distilled water to prevent the gels from drying out. Store the gels at 4°C to 8°C in a closed container, protected from light.

3 Electrotransfer instructions

Step	Action
1	Fill up the tank with transfer buffer to the **DIGE Buffer 4.5 L** fill line.
2	If the gels were stored at 4°C, equilibrate the gels to room temperature.
3	Equilibrate the membrane: **a.** Incubate the membrane for 5 minutes in 100% methanol on an orbital shaker or rocking platform set to gentle agitation. **b.** Incubate the membrane in transfer buffer (Tris 25 mM, Glycine 192 mM, Methanol 20% (v/v)) for 15 minutes on an orbital shaker or rocking platform set to gentle agitation.
4	For one transfer stack, soak two pieces of TE76 blotting paper and two sponges in transfer buffer.
5	Assemble the first half of the transfer stack: **a.** Place the black half of the transfer cassette on the lab bench next to the sink. **b.** Place one pre-soaked sponge on the black half of the transfer cassette.
6	Take out the gel from the gel cassette: **a.** Cut the silicone sealing on both short sides of the gel cassette with a scalpel. **b.** Place the gel cassette on the lab bench. Make sure that the notched glass plate is facing up. **c.** Gently pry open the gel cassette with a plastic wedge tool. Lift off the notched glass plate. **d.** Remove plastic spacers from the glass plate. **e.** Remove the IPG strip with the plastic wedge tool. **f.** Place one pre-soaked blotting paper on top of the gel. **g.** Use a wet roller to remove any bubbles, and increase the contact between the blotting paper and the gel.
7	Assemble the second half of the transfer stack: **a.** Lift up the temporary stack (glass plate, the gel, and the blotting paper) and turn it over. Place it on the sponge with the blotting paper facing down.

Step	Action

b. Gently, detach the gel from the glass plate with a plastic wedge tool and lift off the glass plate.

Tip:

The position of the gel on the transfer stack can be adjusted slightly by carefully rolling the wet roller on the gel.

c. Place the equilibrated membrane on top of the gel. Use the wet roller to remove any bubbles between the gel and the membrane.

d. Place one pre-soaked blotting paper on the membrane. Remove any bubbles between the layers with the wet roller.

e. Place one pre-soaked sponge on top of the blotting paper.

f. Slide the red half of the transfer cassette into the groove of the black half.

g. Press the black half and the red half of the transfer cassette together. Close the hinge over both halves to lock the transfer cassette.

Placement	Transfer stack
Top	Red half of the transfer cassette
Layer 6	Sponge
Layer 5	Blotting paper
Layer 4	Membrane
Layer 3	Gel
Layer 2	Blotting paper
Layer 1	Sponge
Bottom	Black half of the transfer cassette

8 Insert assembled transfer cassettes into the transfer cassette holder. Make sure that the polarity of the transfer cassette is aligned with the polarity of the transfer cassette holder.

9 Insert the transfer cassette holder in the tank. Make sure that the polarity of the transfer cassette holder is aligned with the polarity of the tank.

Step	Action
10	Fill up the tank with transfer buffer to cover the entire gel in the transfer cassette, or up to the **Transfer Buffer Max Fill** line. ***Note:*** *Do not exceed the* **Transfer Buffer Max Fill** *line when filling the tank with transfer buffer.*
11	Plug in the power leads to the electrophoresis power supply. Make sure that the polarity of the power leads matches the polarity of the electrophoresis power supply.
12	Run the electrotransfer with the following run conditions:

Current (mA)	Time (min)
400[1]	90[2]

[1] The maximum electrical input for the product is 800 V, 400 mA, and 100 W.
[2] Depending on the molecular weight of the proteins in the sample, the running time can require optimization.

13 Process the membrane:

Detection method	Action
Fluorescence scan	a. Air dry the membrane. b. Scan the membrane for fluorescence.
Protein detection, such as DIBE™ coverage analysis	a. Keep the membrane in transfer buffer. b. Follow the instructions from the manufacturer of the protein detection method.

14 For long term storage, air dry the membrane in a box protected from light. Store the dried membrane protected from light.

4 Technical specification

Parameter	Specification
Gel cassette size (W × H × D)	277 × 217 × 7 mm
Gel size (W × H × D)	255 × 186 × 1 mm
Number of gels	1 to 2 gels
Number of transfer cassettes	1 to 2 transfer cassettes
Maximum voltage	800 VDC
Maximum amperage	400 mA
Maximum wattage	100 W
Maximum buffer temperature	35°C[1]
Buffer volume (electrophoresis)	4.5 L anode buffer 1.2 L cathode buffer
Buffer volume (electrotransfer)	Approximately 7.0 L transfer buffer, depending on the number of transfer stacks in place.

[1] When using ethanol in the transfer buffer, make sure that the buffer temperature does not exceed 30°C. Heating flammable liquids above their flash point can cause fire.

WARNING

Shock hazard. Do not set the electrophoresis power supply to an electrical input above 800 VDC, 400 mA and 100 W during any part of the run. Exceeding the electrical input limits can result in fire or electric shock.

CAUTION

Fire hazard. Do not exceed the maximum buffer temperature. Heating flammable liquids above their flash point can cause fire.

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